2827-Pos Board B257

Reconstitution of the Coat Protein Complex II Induces Morphological Changes on Artificial Membranes

Sebastian Daum[1], Daniela Krüger[1], Lea Dietrich[1], Mona Groß[1], Annette Meister[2], Kirsten Bacia[1].
[1]ZIK HALOmem, Martin-Luther-University Halle-Wittenberg, Halle, Germany, [2]Cryo Electron Microscopy Group, Martin-Luther-University Halle-Wittenberg, Halle, Germany.

In eukaryotic cells, the transport of cargo from the endoplasmic reticulum (ER) to the Golgi apparatus is conducted by protein-coated membrane vesicles. The stepwise formation of the coat protein complex II (COPII) on ER membranes is initiated by the small GTPase Sar1. Following GDP/GTP exchange, which is catalyzed by the guanine nucleotide exchange factor (GEF) Sec12, Sar1 undergoes a conformational change upon which an amphipathic helix inserts into the proximal leaflet of the ER membrane. Membrane-bound Sar1p subsequently recruits the inner and outer coat subcomplexes Sec23/Sec24 and Sec13/Sec31 to complete the COPII coat. This process leads to marked changes in membrane curvature. We apply in vitro reconstitution studies with purified yeast proteins on giant unilamellar vesicles (GUVs) as an artificial membrane system to examine the COPII vesicle formation process. Using confocal microscopy for visualization, we observe the formation of rigid tubes protruding from the GUVs under conditions where GTP hydrolysis is prevented. Cryo-electron microscopy shows tubules that apparently fail to fission into separate vesicles. Moreover, we can distinguish individual stages in the formation of the COPII coat on the bilayer. Altering the lipid composition, we observe different membrane morphologies. Our in vitro investigation of the COPII complex shows that GTP hydrolysis is not essential for binding of the coat to the membrane but may have a role in vesicle fission.

2828-Pos Board B258

Solubilization of Membranes by Styrene Maleic Acid (SMA) Results in Formation of Nanodiscs with Retention of Native Lipid Composition

Juan J. Dminguez Pardo.
MBB, University Utrecht, Utrecht, Netherlands.

Purification of membrane proteins has been a real headache for the scientific community during the last decades. Recently, a new purification method has emerged using the SMA (styrene-maleic acid) co-polymer, which is able to solubilize membranes into small bilayer discs. Its importance lies mostly in the presumed ability of retaining the native lipid environment of the membrane proteins, preventing misfolding and/or loss of activity. However, it cannot be excluded that SMA preferentially solubilizes specific lipids, thereby modifying the extent to which the native lipid environment is indeed retained.

To find out whether preferential solubilization by the polymer occurs, we performed an extensive biophysical study using model membranes of synthetic lipids with well-defined compositions. Solubilized and non-solubilized material was obtained by adding limiting amounts of SMA polymer to the phospholipid vesicles. The mixture was centrifuged and lipid analysis was performed on the supernatant, containing the nanodiscs, and the non-solubilized fraction in the pellet. This procedure was performed for mixtures of different phospholipids, in which we varied the acyl chain length, unsaturation, head group charge and lipid phase. The results showed no preferential solubilization in any of these lipid mixtures, the only exception being a raft-like system (DOPC/SM/cholesterol), where it was shown that SMA is incapable of solubilizing SM/cholesterol-rich domains into nanodiscs.

The results highlight the potential of SMA polymer as a strong alternative for membrane protein purification, in which the native membrane lipid composition is generally retained. Interestingly, the behavior of the raft-like systems upon addition of SMA resembles the behavior of detergent-resistant membranes (DRMs) when membranes are treated with detergent, paving the way for novel approaches of lipid raft isolation.

2829-Pos Board B259

The Effect of Oxidized Lipids on the Interplay of Bcl-2 and Bax Proteins at Mitochondrial Membranes

Martin N. Lidman[1], Artur Dingeldein[1], Marcus Wallgren[2], Anders Pedersen[3], Göran Karlsson[3], Sarka Pokorna[4], Martin Hof[4], Gerhard Gröbner[1].
[1]Chemistry, Faculty of Science and Technology, Umeå, Sweden, [2]Medical Biochemistry and Biophysics, Faculty of Science and Technology, Umeå, Sweden, [3]Faculty of Science, Gothenburg, Sweden, [4]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Mitochondria play a crucial role in the intrinsic apoptotic pathway. The Bcl-2 family proteins, which interact with the mitochondrial outer membrane to modulate membrane permeability, are key regulators of this pathway. Intracellular oxidative stress is one major factor leading to apoptosis accompanied by the permeabilization of the mitochondrial outer membrane; a process causing release of apoptotic factors such as cytochrome c. Upon onset of intracellular stress, phospholipids can become oxidized in their unsaturated fatty acid region. These oxidized phospholipids (OxPls) can severely alter the properties of these mitochondrial membranes, and can therefore have i) a direct effect on the membrane properties and its perforation and ii) can have an indirect effect by altering the function of membrane-associated Bcl-2 proteins (such as the anti-apoptotic Bcl-2 and the apoptotic Bax). We therefore devised a model system that mimics oxidative stress conditions by incorporating oxidized phospholipids (OxPls) into mitochondria-mimicking vesicles, and studied the OxPls' impact on Bax-membrane interactions. Conformational changes in the protein upon contact with the lipid membranes were monitored using far-UV circular dichroism (CD) spectroscopy. Differential scanning calorimetry (DSC) and solid-state magic angle spinning nuclear magnetic resonance (MAS NMR) spectroscopy was used to study membrane organization. In a biophysical study combining CD with surface plasmon resonance techniques we also investigated the putative interaction of solubilized full-length human Bcl-2 with Bax. There, we found a direct Bcl-2 interaction with Bax in the presence of sub-CMC concentration of detergent and could observe a high affinity between both partners (KD 35.8 nM).

2830-Pos Board B260

The Interaction of Hsp70 with Phosphatidylserine Membranes is Mediated by a Highly Positive Region of the Molecule

Victor Lopez[1,2], David M. Cauvi[2,3], Nelson Arispe[4], **Antonio De Maio**[2,5].
[1]Initiative for Maximizing Student Development Program, University of California, San Diego, La Jolla, CA, USA, [2]Center for Investigations of Health and Education Disparities, La Jolla, CA, USA, [3]Department of Surgery, University of California, San Diego, La Jolla, CA, USA, [4]Department of Anatomy, Physiology and Genetics, Uniformed Services University, Bethesda, MD, USA, [5]Departments of Surgery and Neuroscience, University of California, San Diego, La Jolla, CA, USA.

Heat shock proteins (hsp) participate in many cellular processes during normal physiological conditions. In particular, they are involved in protein folding and are referred to as molecular chaperones. During stress conditions, hsp participate in the repair and recovery from an insult and confer protection from subsequent stresses. In addition, they are released into the extracellular milieu where they act as signaling molecules directed at activating the immune system to avoid the propagation of the insult. Hsp70, the major inducible form of the hsp family, does not contain any consensus secretory signal that predicts its export via the classical ER-Golgi secretory pathway. The proposed mechanism for the release of Hsp70 requires an initiated translocation into the plasma membrane. However, Hsp70 does not contain any hydrophobic domain that can predict its insertion into lipid membranes. Using an in vitro liposome insertion assay, we have determined that Hsp70 displays selectivity for negatively charge lipids and its insertion is enhanced by a decrease in the membrane fluidity. The region that is inserted into the lipid membrane has been mapped toward the C-terminus end, which contains the peptide binding domain. Lipid recognition/insertion requires a highly positive region on the molecule. Finally, the addition of ADP, but not ATP, reduced membrane insertion, suggesting that the interaction with the lipid bilayer is dependent on the conformation of the molecule.

Supported by NIH R01 GM098455.

2831-Pos Board B261

Elucidating the T Cell Receptor Transmembrane Organization via Multi-Scale Molecular Dynamics Simulations

Antreas C. Kalli[1], Andre Cohnen[2], Oreste Acuto[2], Mark S.P. Sansom[1].
[1]Biochemistry, University of Oxford, Oxford, United Kingdom, [2]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom.

The T cell antigen receptor (TCR) plays a key role in the adaptive immunity of all vertebrates. It is able to identify with high sensitivity, specificity and rapidity peptide antigens from invading microbial pathogens presented by MHC proteins (peptide-MHC) and signals via multiple intracellular pathways to prepare adequate countermeasures to fend them off. The TCR has a complex structural organization consisting of the TCR $\alpha\beta$ heterodimer, which recognizes peptide-MHC, and it is non-covalently associated with three dimers (the CD3 $\varepsilon\gamma$, $\varepsilon\delta$ and $\zeta\zeta$) that regulate signal transduction. Despite advances in the field, the topology of TCR-CD3 subunit organization is still largely hypothetical. Molecular dynamics simulations can now be applied with confidence to study the assembly of transmembrane (TM) segments of membrane proteins in model lipid bilayers. In this study we probe the assembly of the TCR $\alpha\beta$ TM heterodimer and its interactions with the CD3 accessory subunits at both atomistic and coarse-grained resolution using a multi-scale simulation approach. Our results suggest a